using NFκB oligonucleotide detected the shifted band in the nuclear protein extracted from Hp infected GCIY. Western blotting for IκB-α showed the decrease of IκB-α after Hp infection. The cells infected with Hp showed 3.2folds relative luciferase activity compared to the cells that were not. 100nM wortmannin activated the luciferase activity by 2.1folds. This suggests that the inhibition of PI3K induces Cdx2. Treatment with 100μM cilostazol suppressed the luciferase activity to half value of the cells without the treatment. These results suggest that Hp infection induces Cdx2, and that PTEN and NFκB plays an important role in its expression. Conclusion: Hp infection induced Cdx2 in GCIY through PTEN-PI3K/Akt-NFκB pathway. This may explain the formation of IM after Hp infection in the human stomach.

W906

H.pylori Induces a PYK2/SHP2 Complex that Modulates Normal Human Gastric Mucous Epithelial Cell Morphology

Kathy Bacon, Rebecca Purice, Brett Sheppard, Clifford Deveney, Michael Rutten

Background: *Helicobacter pylori* (Hp) adherence to gastric epithelial cells activates several signal transduction pathways. In preliminary data we found that Hp adherence to gastric cells increased intracellular Ca^{2+} $[Ca^{2+}]_i$ levels (*Am J Physiol*, 285:G163-76, 2003), and increased $[Ca^{2+}]_i$ can activate the non-receptor proline-rich tyrosine kinase-2 (PYK2). In other cell types, both PYK2 and the phosphatase SHP2 (SH2 containing protein tyrosine phosphatase-2) are involved in regulating cell morphology, proliferation, or apoptosis. We therefore wanted to examine in the present study the relationships between PYK2, SHP2 and cell morphology in normal human gastric mucous epithelial cells. **Methods**: Normal human gastric mucous epithelial cells from surgical specimens were cultured in DMEM serum media. Hp wild-type strains (60190, 84-183) were grown on agar plates. Immunoprecipitation and Western blotting was used to detect phosphorylation of PYK2, SHP2, and paxillin. Immunofluorescence microscopy was used to examine signaling molecules and cell morphology. **Results**: In Hp treated cells, a rapid 3-4 fold increase in phosphorylated PYK2 was detected within 10 min that was paralled by a ~2-fold increase in paxillin phosphorylation. In Hp-treated cells, PYK2 became dephosphorylated after 10 min which was paralleled by a ~2.3-fold increase in SHP2 phosphorylation. In Hp treated cells, anti-pPYK2 and anti-pSHP2 immunoprecipitations detected the phosphorylated forms of PYK2, SHP2, and paxillin. Pretreatment of cells with the intracellular Ca^{2+} chelator BAPTA (10uM) or PKC inhibitor GF-109203X (5uM) reduced PYK2 and paxillin phosphorylation. No PYK2 or SHP2 phosphorylation was observed with heat-killed bacteria. Long term (4hr-24hr) Hp-treatment of cells produced cell rounding and detachment. Pretreatment of cells with the SHP2 inhibitor, calpeptin, prolonged Hp-induced PYK2 phosphorylation and attenuated the morphological responses. Immunofluorescence of Hp-treated cells detected PYK2/SHP2 primarily at focal adhesions with paxillin. **Conclusions**: 1) *H. pylori* treatment of normal human gastric mucous epithelial cells induces phosphorylation of PYK2 and paxillin, and the binding of the phosphatase SHP2 to PYK2 may regulate PYK2 dephosphorylation; 2) *H.pylori* activation of PYK2/SHP2 may be part of a signaling mechanism in the control of focal adhesions and paxillin for regulating normal gastric epithelial cell morphology, proliferation, or apoptosis.

W907

Cyclooxygenase-2 Gene Disruption Enhances Gastric Inflammation But Inhibits Gastric Epithelial Proliferation Induced by H. pylori Infection

Qingqing Li, Harry H. X. Xia, Minhu Chen, Jun Zhong Peng, Qing Gu, Jiantao Cui, Chi-Hin Cho, S. K. Lam, Marrie C. M. Lin, Douglas E. Berg, Zhehui Feng, Robert Langenbach, H. F. Kung, Benjamin C. Y. Wong

Background & Aims: Cyclooxygenase (COX) is a rate-limiting enzyme for prostaglandins, which play an important role in inflammation and carcinogenesis. COX-2 is induced by a variety of factors including cytokines, growth factors and tumor promoters. The aim of this study was to determine the effect of COX-2 on H. pylori-induced gastric inflammation, apoptosis and cell proliferation. Methods: H. pylori strain TN2 was inoculated into the stomachs of strain C57/DBA1 of wild-type (WT) and COX-2 deficient (COX-2-/-) mice, and the mice were sacrificed 24 weeks later. WT and COX-2 deficient mice without H. pylori inoculation were used as controls. The density of H. pylori colonization (by culture), gastric COX-2 protein expression (by Western blotting), gastric prostaglandin E2 (PGE2) levels (by EIA), the severity of chronic gastric inflammation, apoptosis (by TUNEL) and proliferation (by Ki-67 assay) of gastric epithelial cells were determined. Results: They were no significant difference in the density of H. pylori colonization between COX-2 deficient and WT mice. COX-2 protein expression was increased in H. pylori-infected WT mice. There was no COX-2 protein expression in COX-2 deficient mice. Gastric PGE2 levels and gastritis scores were increased in WT and COX-2 deficient mice with H. pylori infection compared with uninfected mice. Without H. pylori infection, COX-2 deficient mice had higher apoptosis index than WT mice. H. pylori infection induced stronger gastritis and apoptosis, but less cell proliferation in COX-2 deficient mice than in WT mice (Table). Conclusion: In 24 week H. pylori colonization study model, COX-2 deficiency enhances gastritis and potentiates the Ai/Pi ratio induced by H. pylori infection.

	Hp- wild-type(n=6)	Hp+ wild-type(n=17)	Hp- COX-2-/- (n=6)	Hp+ COX-2-/- (n=10)
Colonization (10⁵ cfu/g)	0	1.9+/-0.8*	0	1.8+/-0.9*
Gastritis score	0.2+/-0.5	1.0+/-0.4*	0.2+/-0.5	1.9+/-0.9*^
PGE2 (pg/mg)	22.8+/-5.0	35.6+/-11.7*	26.7+/-3.8	37.2+/-6.9*
Apoptosis index	13.4+/-13.7	103.6+/-47.9*	109.2+/-60.8	144.0+/-59.7^
Proliferation index	16.8+/-6.7	39.0+/-2.2*	18.5+/-8.5^	19.3+/-7.5^

*P< 0.05, vs. uninfected mice; ^P< 0.05, vs. H. pylori (Hp)+ wild type mice

W908

Surfactant Protein D, a Collectin in Gastric Epithelial Cells, Prevents Colonisation at Low Infective Dose of *Helicobacter felis* in Mice

Wafa Khamri, Mubagir Walker, Amy Anderson, Marjorie Walker, Howard Clark, Mark Thursz

Aim: *Helicobacter* species establish persistent infection in gastric mucosa. Surfactant Protein D (SP-D), a collectin of the innate immune system, is expressed in gastric mucosa in vivo and is upregulated in *Helicobacter* infection. *In vitro* SP-D binds to *H. pylori* and *H. felis* causing aggregation of bacteria. The aim of this study is to determine the role of SP-D in host defence in gastric colonisation by *Helicobacter* and gastric inflammation and immunity in the SPD deficient mouse model. Methods: Specific pathogen-free SP-D -/- mice (n = 30, C57BL/6 background) and C57BL/6 (n = 30) wild type mice were infected by gavage with 10^1 or 10^3 *H. felis* for 3 weeks to determine the ability to clear the infection at low infective doses and 10^7 (3x) *H. felis* for 12 weeks to further investigate the inflammatory response. Gastric colonisation by *H. felis* was assessed using histology and real-time PCR. T cell proliferative response for *H. felis* specific antigens was examined by ^{3}H-thymidine incorporation assay. Inflammatory scores were determined by neutrophil counting in four areas of the stomach (cardia, body, transitional zone and antrum). Results: SP-D -/- mice were effectively colonised by *H. felis*. By 3 weeks post-challenge with 10^3 infective dose, SP-D -/- mice showed a significantly higher colonisation rate to that of the controls (p = 0.04). The T cell proliferative response was also significantly reduced in the absence of SP-D (p = 0.001). Neutrophil responses were not significantly different between SPD-/- and wild type mice. Conclusions: The absence of SP-D is associated with increased susceptibility to low doses of infection, failure in bacterial clearance and reduced levels of T cell response to *Helicobacter*. This suggests that SP-D has a role in protecting against infection with small innocula of the pathogen and is involved in activating or increasing antigen presentation to antigen presenting cells.

W909

Differential Regulation of Antimicrobial Peptides via Toll-Like Receptors in Helicobacter Pylori Gastritis

Jan Otte, Heike Neumann, Rainer Lebert, Andreas Bastian, Henning Schrader, Wolfgang E. Schmidt, Frank Schmitz

Introduction and Aim: Antimicrobial peptides are effector molecules of the innate immune system and are crucial in gastrointestinal barrier function with Toll-like receptors being pattern recognition receptors in the initiation of such immune responses. The aim of the study was to systematically characterize the expression of various antimicrobial peptides in infectious and non-infectious gastritis and to clarify a putative role of Toll-like receptors (TLRs) as well as involved signaling mechanisms. Methods: Gastric biopsies were obtained from patients with informed consent (n = 55; 12 Helicobacter pylori (Hp) positive gastritis, 9 C-gastritis, 5 unspecific gastritis, 23 patients with PPI-therapy, 6 controls without any inflammation or treatment). Gastric AGS cells were incubated with cagA and vacA positive and negative H. pylori strains or pro-inflammatory stimuli. Expression of antimicrobial peptides was quantified by real-time PCR. Intracellular signaling was determined by Western blot analysis and specific blockage of distinct signaling molecules. To clarify a putative role of TLRs basal expression patterns were determined and transient transfection of TLR performed. Results: The gastric mucosa constitutively expresses β-defensin 1 (hBD-1) while expression of hBD-2 is induced in both infectious and non-infectious gastritis. Expression of HD-5 and hBD-4 significantly correlated with infection by H. pylori. Expression of antimicrobial peptides RNase 7 and LL-37 was detected in samples of healthy controls as well as in various forms of gastritis with no obvious correlation to infection with Hp. Expression of α-defensin 6 (HD-6), hBD-3, hBD-5 and hBD-6 was not detected. AGS cells constitutively express hBD-1, whereas stimulation with living cagA and vacA positive Hp and Hp lysates but not Hp conditioned media differentially induced the expression of HD-5, hBD-2 and hBD-4. Expression of antimicrobial peptides is induced following activation of TLRs (2 and 5) and is mediated by the MAP kinase ERK1/2 but not p38. Conclusion: Antimicrobial peptides are differentially expressed in infectious and non-infectious gastritis in humans. Induction of peptide expression by H. pylori requires direct contact with the microorganism but not additional mediators. Recognition of H.pylori and initiation of specific effector molecules seems to be mediated by TLRs. These results suggest, that differential innate immune responses contribute to the protection of gastral mucosa.

W910

A Functional Toll-Like Receptor 4 Polymorphism Increases the Risk of Gastric Cancer

Georgina L. Hold, Malcolm G. Smith, Wong-Ho Chow, Charles S. Rabkin, Joseph F. Fraumeni Jr., Emad M. El-Omar

Background: Host genetic factors are emerging as key determinants of clinical outcome of *H. pylori* infection including gastric cancer (GC). Toll-like receptor 4 (TLR4) is an important pattern recognition receptor that is key to eliciting an inflammatory response against bacterial lipopolysaccharide (LPS). We have recently shown that a functional missense mutation (Asp299Gly) in the fourth exon of the *TLR4* gene, which causes aberrant LPS handling, increases the risk of hypochlorhydria and premalignant changes in the stomach. Aim: We examined whether the *TLR4* Asp299Gly polymorphism influences risk of gastric cancer (GC) in Caucasians. Subjects & Methods: We used PCR-RFLP and 5' nuclease assays to genotype the *TLR4* Asp299Gly polymorphism in a Polish population-based case-control study comprising 360 gastric cancer cases and 420 controls. Odds ratios and 95% confidence intervals (CI) were calculated and logistic regression was used to adjust for confounding variables. Results: The frequency of the mutation in the control population was similar to other Caucasian populations (7%) and the alleles were in Hardy-Weinberg equilibrium. There was a significant association between carriage of the Asp299Gly mutation and increased risk of GC (adjusted OR = 2.2, 95% CI: 1.4-3.7). The increased risk applied equally to diffuse and intestinal type GC and was stronger for the non-cardia subsites. The risk was higher

for *H. pylori* positive cases. Conclusions: Our results indicate that carriage of the *TLR4* Asp299Gly mutation increases risk of GC in this Caucasian population. We speculate that aberrant handling of LPS (of any origin) due to this mutation leads to an exaggerated inflammatory response that is characterised by severe gastritis, gastric atrophy, hypochlorhydria, and ultimately increased risk of GC. Our findings expand and strengthen the role of host genetic factors in the pathogenesis of *H. pylori*-induced GC.

W911

Presence of B7-H1 on Gastric Epithelial Cells and Its Potential Role in Regulating T Cells During Helicobacter Pylori Infection

Soumita Das, Giovanni Suarez, Ellen Beswick, David Bland, Victor Reyes

Background and aim: Gastric epithelial cells (GEC) express class II MHC and co-stimulatory molecules such as CD86 (B7.2), which may permit them to act as antigen presenting cells (APC). Since they are strategically located between luminal antigens and resident intraepithelial and lamina propria T cells, GEC could play a major role in the immunopathogenesis associated with H. pylori infection. However, during H. pylori infection the T cells are hyporesponsive and do not afford protection. The ligand PD-L1 (B7-H1) on APC binds with the T cell receptor PD-1 that negatively regulates T cells. Thus, the hypothesis addressed in this study is that GEC express B7-H1 that allow them to inhibit local T cells and contribute to the chronicity of H. pylori infection. Methods: The presence of new B7 members was detected by RT-PCR. Changes in the expression of B7-H1 (PD-L1) by GEC following H. pylori infection were determined by real time quantitative RT-PCR and flow cytometric analysis. The functional role of B7-H1 in the crosstalk between GEC and T cells was assessed by co-culturing them in the presence of blocking agents such as antibodies to PD1, B7-H1, ICOS-Fc, B7-H1Fc and PD1Fc. Results: In addition to the co-stimulator ligand ICOSL, RT-PCR revealed the expression of PD-L1 and PD-L2 by GEC. PD-L1 was also detected by flow cytometry in GEC such as Kato III, N87, AGS cell lines and also in nontransformed HS738 cells. PD-L1 expression by GEC increased following H. pylori infection as detected by real time RT-PCR. Interestingly, IFN gamma which is produced during H. pylory infection increased PD-L1 expression by GEC. TNF-alpha also increased PD-L1 expression whereas LPS and IL4 had very little effect in PD-L1 expression. Coculturing GEC with CD4+ T cells revealed the functional role of PD-L1 in promoting T cell apoptosis, in regulating Th1/Th2 cytokine expression and limiting T cell proliferation. Conclusions: Induction of PDL1 in H. pylori treated GEC indicates the possible role of PD-L1 in immunosuppression. Functional studies indicate the involvement of other non-PD1 receptor or other B7-H members in GEC.

W912

Helicobacter Pylori Infection Suppresses the Expression of Gastric Ghrelin

Asish Bassi, Helina Kamarova, Lazlo Pazmany, Keith Bodger

Background: Ghrelin is a recently described growth hormone releasing peptide synthesised mainly in the oxyntic mucosa of the stomach. It has a powerful effect on the secretion of growth hormone and directly signals the hypothalamic regulatory nuclei that control energy haemostasis. H. pylori gastritis is invariably associated with a local immune response though the organism generally persists and leads to chronic gastric inflammation. The influence of H. pylori associated gastritis on the regulation of gastric ghrelin has not been studied. We speculate that inflammation of the oxyntic mucosa may impair the ghrelin synthesis. This may account for the association of H. Pylori infection with growth delay in childhood. Methods: Total RNA was extracted from a pair of fundic biopsies. This was reverse transcribed and cDNA was subject to quantitative real time PCR with results normalised to the housekeeping gene hHPRT. H. pylori status was determined by separate antral biopsies. The Ghrelin sequence used was: Fwd:5-ACAACTCCTTGCAGCTCC-3 ;Rev:5-ATCTTCATGAAGG-TAGRCAGTC-3. Statistical analysis was done using Mann Whitney Test. Results: Twenty six patients were recruited for the study, of which five patients had H. pylori gastritis and the rest had normal histology. There was no significant difference in demographics and anthropometrics in the two groups [H. pylori gastritis v Controls; Mean (SD); Age - 48(11) v 47(14), p = 0.7; Sex (M: F) - 4:5 v 7:10; BMI - 28.5(3) v 27.7(4), p = 0.5 and bioimpedence - 473(85) v 512(85), p = 0.3]. The Ghrelin/hHPRT mRNA ratio [mean (SD)] was 0.007(0.008) in samples with H. Pylori gastritis compared to 0.038(0.04) in controls (p = 0.01). Conclusion: H. pylori infection is associated with down regulation of gastric fundic gene expression. Reduced gastric production of ghrelin in patients with H. pylori associated gastritis may contribute to extra intestinal manifestations of the infection via reduced GH release and/or via disturbances in the regulation of appetite and energy homeostasis. The mechanism by which the local inflammation inhibits gastric ghrelin expression still remains to be investigated.

W913

Helicobacter felis Induces Hypergastrinaemia, ECL Cell Hyperplasia and Gastric Carcinoids in Mongolian Gerbils

Jean E. Crabtree, Anthony H. T. Jeremy, Andrea Varro, Michelle Court, Philip A. Robinson, Michael F. Dixon

Introduction: Short-term studies have shown that *H. felis* infects the Mongolian gerbil causing gastritis, but the pathological effects of long-term *H. felis* infection in gerbils are unknown. Aims: To evaluate pathology induced by *H. felis* in the gerbil and to compare the effects of *H. felis* and *H. pylori* SS1 strain in this model. Methods: Male gerbils were orally challenged with *H. felis* (*Hf*) or *H. pylori* (*Hp*) SS1 strain. Infected animals (n = 33) plus controls (n = 17) were sacrificed at 36 and 62 weeks post-infection (p.i.). Infection was confirmed by culture and/or histology. Serum gastrin was measured by radioimmunoassay. Haematoxylin and eosin, and anti-chromogranin stained sections were used to grade gastric pathology and enterochromaffin-like (ECL) cells respectively. Results: All *Hf* and *Hp* inoculated gerbils were infected. Gastric pathology with *Hf* at 62 weeks p.i. was greater in the corpus than the antrum, consisting of marked atrophy of parietal/chief cells, cystic changes and mucous metaplasia. In the antrum at 62 weeks, *Hp* was associated with significantly greater chronic inflammation ($p < 0.05$), polymorph activity ($p < 0.005$) and atrophy ($p < 0.003$) than *Hf*. In the corpus no significant differences in chronic inflammation and atrophy were observed but *Hf* was associated with significantly greater activity ($p < 0.05$) and ECL cell hyperplasia ($p < 0.01$) than *Hp*. At 62 weeks p.i. serum gastrin was significantly increased in *Hf* (138 ± 57.2 pM, $p < 0.001$) but not *Hp* infection (42.3 ± 13.6;$p = 0.09$) compared to uninfected controls (10.3 ± 1.8). Gastric carcinoids were present in 3/15 *Hf* infected gerbils but absent in 18 *Hp* SS1 strain infected gerbils. Conclusions: Gastric pathology induced by *Hp* SS1 strain and *Hf* in the gerbil differs. Long-term *Hf* infection results in corpus predominant gastritis, elevated gastrin, ECL cell hyperplasia and gastric carcinoids.

W914

Treatment with Gastrin and Hypergastrinemia Enhance the Functional and Pathological Impairment Induced by Helicobacter Pylori (Hp) Infection in Mongolian Gerbils

Tomasz M. Brzozowski, Peter C. Konturek, Zbigniew Sliwowski, Danuta Drozdowicz, Stanislaw J. Konturek, Jerzy Stachura, Agata Ptak-Belowska, Wieslaw W. Pawlik, Eckhart G. Hahn

Hp-infection in Mongolian gerbils is an established experimental model of gastric carcinogenesis resulting from the long-term infection of gastric mucosa by this germ but the role of gastrin or prolonged hipergastrinemia induced by omeprazole treatment in these animals remians to be established. We studied the effects of intragastric inoculation of Mongolian gerbils with Hp strain ($cagA^+$ $vacA^+$, $5x10^6$ CFU/ml) isolated from gastric ulcer patient. Hp-infected and non-infected gerbils received daily treatment for the 3 weeks with: 1) vehicle (saline); 2) gastrin-17 (10 nmol/kg i.p.); 3) pentagastrin (40 nmol/kg s.c.) and 4) omeprazole (10 mg/kg s.c.). At 4, 12, 30 and 60 wks upon Hp inoculation, the morphological changes in glandular mucosa were assessed by histology, the viable Hp analyzed by rapid urease test and density of Hp-colonization was evaluated by counting of the number colonies per plate. In addition, gastric blood flow (GBF) was measured by H_2-gas clearance technique, plasma gastrin was determined by RIA and expression of COX-2 protein was assessed by Western Blot. The Hp infection was detected in all animals by histology, Hp culture and rapid urease test. Early lesions were seen 4 wks upon Hp-inoculation and consisted of chronic gastritis with increased mucosal foldings and elongated interfoveolar ridges and formation of multiple lymphoid follicles in the gastric mucosa. By the end of study typical adenomateous hyperplasia with cellular atypia was observed together with atrophic gastritis, intestinal metaplasia, dysplasia and intraepithelial neoplasia, especially in gerbils treated with gastrins or omeprazole. In Hp-infected gerbils, plasma gastrin levels was increased by 4-5 folds and the significant rise in the mucosal protein expression of COX-2 were observed and these effects were markedly enhanced in gerbils treated with gastrin-17, pentagastrin and omeprazole. The GBF in Hp-infected gerbils treated with gastrin-17, pentagastrin or omeprazole was significantly lower than that in Hp-infected treated with vehicle and this decrease in GBF remained constant until the end of observation period. We conclude that gastrin and prolonged hypergastrinemia caused by long omeprazole treatment with its subsequent COX-2 induction play a major role in the development of typical functional changes such as impairment of gastric mucosal microcirculation and by promoting of pathological changes leading to gastric carcinogenesis.

W915

Helicobacter Pylori Infection of the Human Antral Cell Line, NCI-N87, Alters Expression Levels of the Cell Matrix Proteins Dystroglycan and Paxillin, Resulting in Increased Rates of Migration

Jian Kang, Victoria S. Conlin, Susan Curtis, Ying Zhao, Edwin Moore, Steven Pelech, Alison Buchan

AIM: Wound-closure requires epithelial cells to migrate as a sheet, as opposed to single cells. We predict that this is achieved by altering the expression of cell matrix proteins whilst cell:cell adhesion protein expression remains constant. Dystroglycan is one of the proteins involved in maintaining the attachment of epithelial cells to the basement membrane. These experiments addressed the question of whether Helicobacter pylori infection alters the expression of attachment proteins dystroglycan and paxillin and cell migration rates. METHODS: Cells were starved for 24h prior to infection with wild type (cag A+/vac A+) H. pylori and incubated for a further 24h. For migration assays a wound was made across the centre of the well and the rate of wound closure monitored at 12h intervals over a 48h period. Cells were lysed in the presence of protease inhibitors for immunoprecipitation experiments or fixed in paraformaldehyde for immunocytochemical analysis using deconvolution microscopy. RESULTS: Western blot analysis of H. pylori infected NCI-N87 cells revealed a decrease in dystroglycan protein expression in conjunction with an increase in both paxillin protein expression and tyrosine phosphorylation (12.6% above control, n = 3). Parallel immunocytochemical analysis showed translocation of dystroglycan and paxilin away from the basal membrane that was correlated with increased cell migration (28.6% above control, n=4). CONCLUSION: H. pylori infection increases the migratory rate of epithelial cells, in part, by decreasing dystroglycan levels and altering the distribution of paxillin in focal adhesions. Funded by the Canadian Institutes of Health Research

W916

Regulation of Host Innate Immune Responses During *H. pylori* Infection

Parjeet K. Boughan, John T. George, Richard Argent, John Atherton, Mona Bajaj-Elliott

Background: Beta-defensins are a family of endogenous anti-microbial peptides that operate in host defence most prominently at mucosal epithelia and skin. We and others have previously shown human beta-defensin (hBD)-2 and -3 to be potent bactericidal agents against *H. pylori*. At present the identity of signalling pathways involved in host-bacterial cross talk leading to modulation of defensin expression remain largely unknown. In the present study we are investigated the role of NFkB and MAP Kinase signalling events, which